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Chrysophylum albidum fruit juice reverses erythrocytes ethylene glycol-induced toxicity in male Wistar rats

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ABSTRACT

In vivo antioxidant properties of Chrysophyllum albidum leaves has been reported, but studies on C. albidum fruit juice are scarce. In the current study, we investigated the antioxidant potential of C. albidum fruit juice on ethylene glycol-induced oxidative stress on the erythrocytes. Experimental design involved twenty five (25) male Wistar rats randomly divided into five (5) study groups. Group 1 was administered with normal saline (Control), groups 2-4 were poisoned with ethylene glycol and treated with 0.2, 0.4 and 0.6 ml of C. albidum fruit juice respectively, and group 5 was poisoned with ethylene glycol, but not treated with C. albidum fruit juice. The results indicated that while there was a significant elevation ($p < 0.05$) in the level of lipid peroxidation product (MDA) in the group poisoned with ethylene glycol alone without treatment, lipid peroxidation levels were restored in the ethylene glycol poisoned rats that were treated with Chrysophyllum albidum fruit juice compared with the control. In addition, there were increases in the activities of glucose-6-phosphate dehydrogenase and catalase in all the groups except in the untreated group poisoned with ethylene glycol alone, where the enzymes activities were significantly ($p < 0.05$) reduced. We conclude therefore that C. albidum fruit juice possesses potent antioxidant property.

Key Words: *Crysophyllum albidum*; Ethylene glycol; Oxidative stress; Erythrocytes; Lipid peroxidation

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INTRODUCTION

Reactive oxygen species (ROS) are involved either in the initiation or progression of carcinogenesis by inducing oxidative stress [1-2]. Also, peroxides and superoxide anions (O^-) produce cytotoxicity/genotoxicity in cellular system. The ROS as well as nitrogen species are formed regularly in the human body and the endogenous antioxidant defenses may not be sufficient to counteract them completely. The hypothesis that oxidative damage to lipids, DNA and proteins contribute to the onset of cardiovascular disease, cancer and neurodegenerative diseases has been widely reported [3].

Ethylene glycol (EG) is a bitter and sweet-tasting dihydric alcohol ($HO\text{-}CH_2\text{-}CH_2\text{-}OH$) that leaves slightly warm sensation in the mouth after ingestion. It is odourless, colourless, insoluble in water, and it possesses antifreeze property. Ethylene glycol is found in many agents such as brake fluids, antifreeze and industrial solvents. Ingestion of EG may result in serious poisoning. Apart from occupational exposure, adults may be exposed to it through suicide attempts, and as a cheap substitute for ethanol. Children also may be exposed to it through accidental ingestion caused by decantation of EG in unlabelled bottles [4]. Although EG itself has a low toxicity, however, *in*

vivo break down by alcohol dehydrogenase, produces four metabolites such as glycoaldehyde, glycolic acid, glyoxylic acid and oxalic acid [5]. These metabolites are cell toxins that can surpress oxidative metabolism, thereby causing central nervous system depression, and cardio-pulmonary and renal failure [6-7]. The rate-limiting step in the metabolism of EG is the conversion of glycolic acid to glyoxylic acid which therefore results in the accumulation of glycolic acids, causing severe acidosis, leading to oxalate precipitating as calcium oxalate in the kidneys, and other tissues [7, 8]. Case reports of EG poisoning have been reported [4].

The search for natural antioxidants that can reverse toxicity induced by toxicants is ongoing. *In vivo* and *in vitro* antioxidant properties of *Chrysophyllum albidum* (CA) leaves have been reported. CA is in the Sapotaceae family native to the Central, Eastern and Western Africa [9] and distributed in Uganda, Niger, Nigeria, Cameroun and Cote d' Ivoire [10]. Sometimes, it is called the white star apple. In Nigeria, it is distributed throughout the Southern part of the country [11]. The fruit is known as 'agbalumo' and 'Udara' in the South Western and Eastern Nigeria respectively. This research was carried out in order to assess the ameliorative effects of *Chrysophyllum albidum* juice on ethylene glycol-induced oxidative stress in male Wistar rats.

MATERIALS AND METHODS

Chemicals

Tris HCl, magnesium chloride, nicotinamide adenine dinucleotide phosphate, monosodium salt, (NADP), glucose-6-phosphate, glycine and bovine serum albumin used in this work were supplied by Sigma Chemical Co. (St. Louis, MO, USA).

Animals and animal care

Animal studies were carried out after the approval of institutional animal care, and use committee. Wistar rats weighing between 230-250 g were obtained from the Animal House Facility of the College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria, where this research was conducted. The animals were housed for one week prior to experiment under controlled conditions with 12 hours light/dark cycle, temperature $22\pm2^{o}C$, and free access to feed and water.

Plant material

The *Chrysophyllum albidum* fruit was obtained from Covenant University Cafeteria 2, Ota, Ogun state, Nigeria. The juice used for this experiment was extracted using muslin cloth and stored in the refrigerator until required for use.

Induction of ethylene glycol toxicity and plant treatment

Ethylene glycol toxicity was induced in overnight fasted rats by providing the rats with 0.75% ethylene glycol.

Experimental Design

In the experiment, twenty five (25) rats were divided into five (5) groups of 5 rats each and grouped as follows:
Group 1: Control rats administered with normal saline.
Group 2: ethylene glycol poisoned rat treated with 0.2 ml of Chrysophyllum albidum fruit juice
Group 3: ethylene glycol poisoned rat treated with 0.4 ml of Chrysophyllum albidum fruit juice
Group 4: ethylene glycol poisoned rat treated with 0.6 ml of Chrysophllum albidum fruit juice
Group 5: rats poisoned with ethylene glycol alone

After 10 days of treating the poisoned rats with *Chrysophyllum albidum* juice, the rats were sacrificed.

Collection of blood samples and preparation of erythrocytes

Blood samples were collected by cardiac puncture into heparinised tubes under mild anaesthesia with diethyl ether and centrifuged at 3,000 rpm for 10 minutes to obtain plasma and erythrocytes. The erythrocytes were suspended in phosphate buffered saline (0.9% NaCl in 0.01 M phosphate buffer, pH 7.4), and centrifuged. This process was repeated twice.

Catalase

The method of Aebi (1984) [12], was used to measure catalase activity. The reaction was started by addition of 0.3 ml of 30 mM hydrogen peroxide (H_2O_2) to 0.65 ml of 50 mM potassium phosphate buffer and 50 µl of the sample. The H_2O_2 decomposition was monitored at 240 nm, 37 °C for 3 min. The catalase activity was expressed as micromoles of H_2O_2 consumed per minute per milligram of sample protein using the molar absorption coefficient of 36 $M^{-1}cm^{-1}$.

Glucosse-6-phosphate dehydrogenase (G6PD)
G6PDH activity was assayed based on spectrophotometric measurement of NADPH formation rate, which is proportional to the G6PD activity [13].

Assay of erythrocytes lipid peroxidation
The level of MDA in the erythrocytes was determined according to the method of Buege and Aust (1978) [14]. In brief, to 2 ml of the sample, 2 ml of 20% trichloroacetic acid was added and heated in boiling water for 60 min and cooled immediately. The mixture was centrifuged and the absorbance of the supernatant measured spectrophotometrically at 532 nm. The lipid peroxidation was calculated using the molar extinction coefficient of malondialdehyde (MDA) (1.56 X 10^5 $M^{-1}cm^{-1}$).

Statistical Analysis
Values were expressed as mean ± standard error of mean (SEM). The homogeneity of data among the groups was tested using Analysis of variance (ANOVA). Where heterogeneity occurred, the groups were separated using Duncan Multiple Range test.

RESULTS AND DISCUSSION

While there was a significant elevation ($p < 0.05$) in the level of lipid peroxidation product (MDA) in the group poisoned with ethylene glycol alone without treatment, lipid peroxidation levels were restored in the ethylene glycol poisoned rats that were treated with *Chrysophyllum albidum* fruit juice compared with the control (Fig 1). In addition, there were increases in the activities of glucose-6-phosphate dehydrogenase (Fig. 2) and catalase (Fig. 3) in all the groups except in the untreated group poisoned with ethylene glycol alone, where the enzymes activities were significantly ($p < 0.05$) reduced.

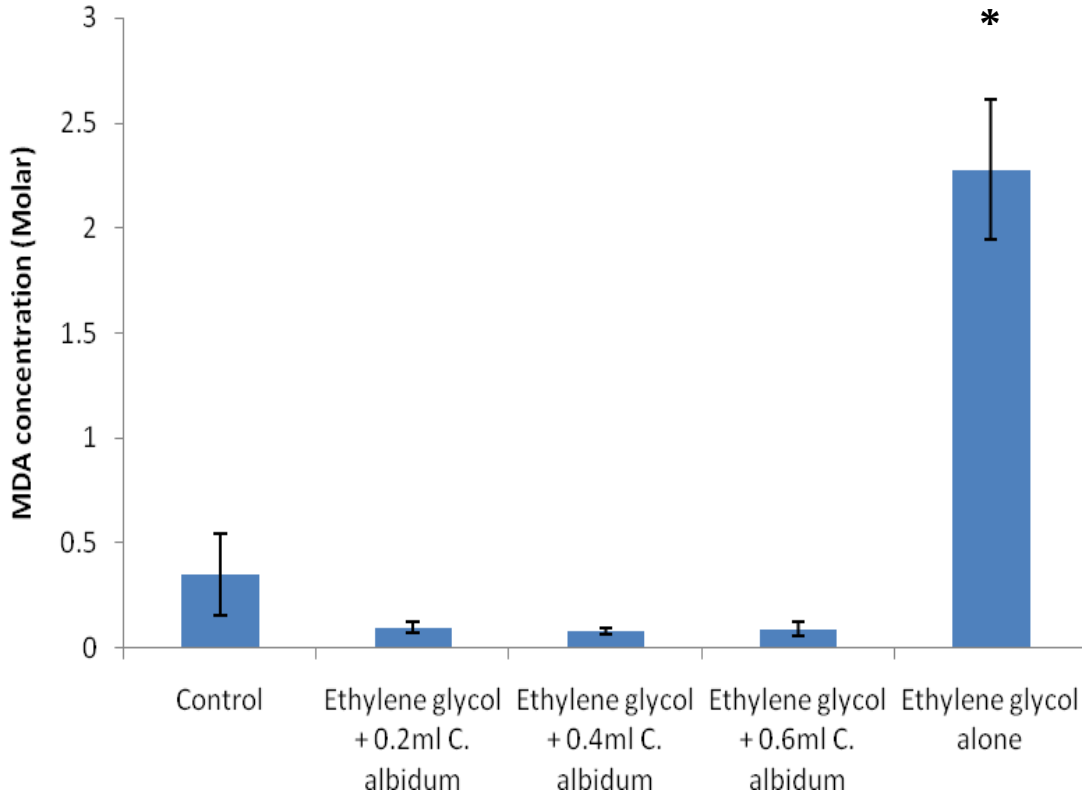

Fig. 1. Effect of ethylene glycol on erythrocytes lipid peroxidation product (MDA) in male Wistar rats.
*Values are expressed as Mean±SD of 5 determinations. *Significantly different from control at p < 0.05.*

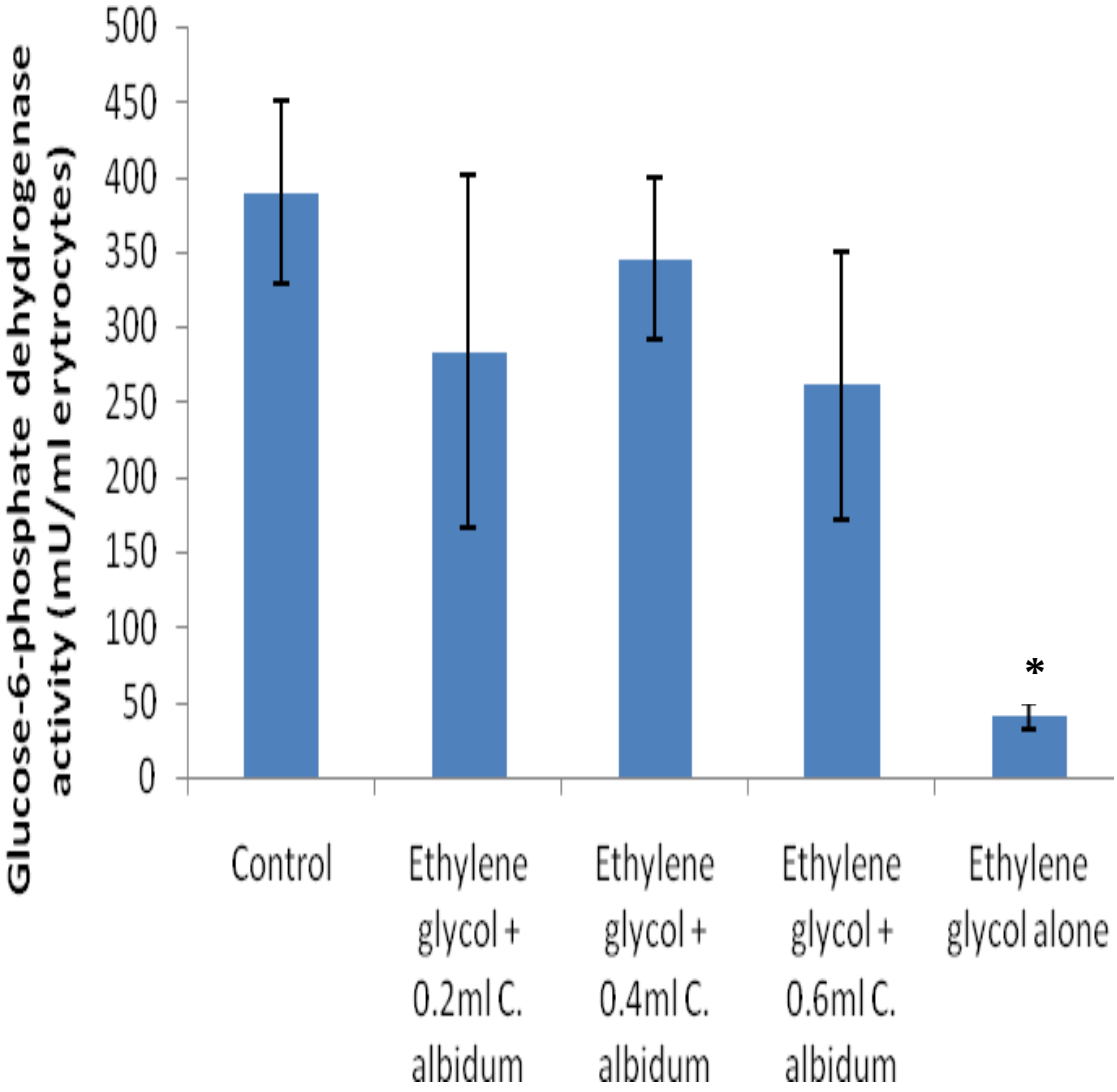

Fig. 2. Effect of ethylene glycol on erythrocytes Glucose-6-Phosphate Dehydrogenase activity in male Wistar rats
*Values are expressed as Mean±SD of 5 determinations. *Significantly different from control at p < 0.05.*

Erythrocytes are sensitive to oxidative damage to a large extent [15-16]. Their sensitivity is due mainly to the presence of polyunsaturated fatty acids (PUFAs) in their membranes as well as high concentrations of cellular oxygen and haemoglobin (Hb) [17-18]. Consequently, the erythrocytes utilize efficient antioxidant mechanism, in the form of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glucose-6-phosphate dehydrogenase (G6PD), and glutathione-S-transferase (GST) and non enzymatic antioxidant molecules such as glutathione, vitamins C and E, to scavenge reactive oxygen species, thereby maintaining membrane integrity. Increase in the use of EG in industry as antifreeze and domestic consumptions have been observed worldwide.

In the present study, we investigated the ameliorative effect of *Chrysophyyllum albidum* fruit juice on ethylene glycol-induced toxicity in Wistar rats. The increased lipid peroxidation observed when ethylene glycol alone was administered, as evidenced by elevated levels of malondialdehyde, suggests increased oxidative stress in the erythrocytes. This was responsible for the depletion of G6PD, and catalase observed in the study. Glucose-6-phosphate dehydrogenase is an enzyme in the pentose phosphate pathway (PPP) responsible for the protection of erythrocytes from oxidative damage by producing reduced nicotinamide adenine dinucleotide (NADPH), and reduced glutathione. Since the erythrocyte lacks a nucleus, mitochondria and other organelles, PPP is the only biochemical pathway responsible for generating reducing capacity [19-20]. In normal erythrocytes, NADPH is regenerated by G6PD during oxidative stress. Impairment of this step prevents reduced glutathione recycling, thereby exposing the erythrocytes to oxidative damage. Because there are no alternative pathways to G6PD-dependent NADPH production in erythrocytes, the erythrocyte is deprived of the opportunity to replace enzyme that

has been lost. Since low levels of G6PD has been linked with accumulation of hydrogen peroxides, [21] it is expected that catalase level will be reduced as observed in this study. Catalase (hydrogen peroxide oxidoreductase, EC 1.11.1.6) is a soluble protein found in the erythrocytes and it protects haemoglobin from peroxidation. Catalase is also important in eliminating the potentially dangerous formation of H_2O_2 in erythrocyte [22]. Reduction in catalase activity therefore, further confirms the oxidative stress reported in this study. The erythrocyte oxidative stress was reversed when *Chrysophyllum albidum* fruit juice was administered together with ethylene glycol, implying that, *Chrysophyllum albidum* fruit juice possesses natural antioxidant properties that can prevent the observed ethylene-glycol induced erythrocyte oxidative stress in the rats. A number of plant products including polyphenolic substances (e.g., flavonoids and tannins) and various plants or crude extracts have been reported to exert potent antioxidant properties [23].

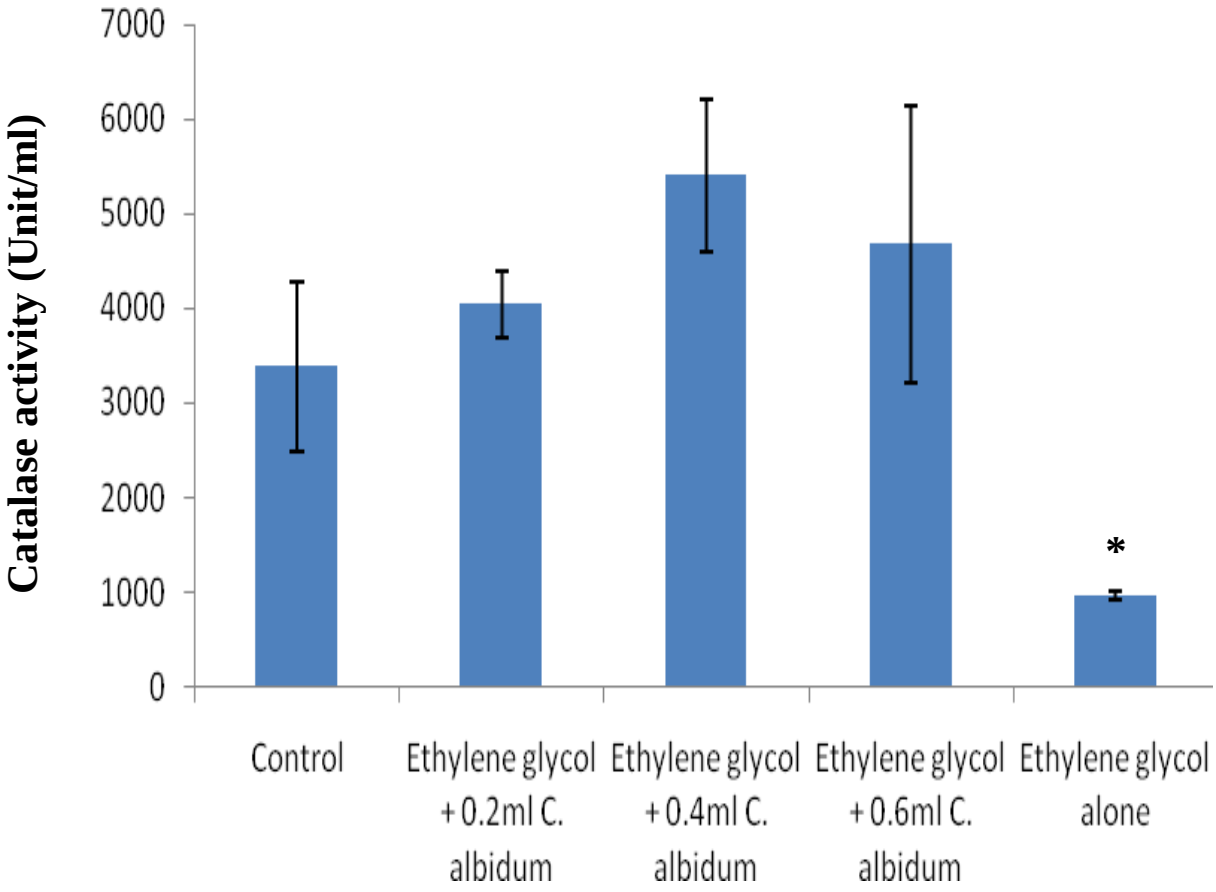

Fig. 3. Effect of ethylene glycol on erythrocytes catalase activity in male Wistar rats
*Values are expressed as Mean±SD of 5 determinations. *Significantly different from control at p < 0.05.*

CONCLUSION

This study has briefly showed that *C. albidum* fruit juice has the potential of reversing EG-induced erythrocytes oxidative stress in male Wistar rats. It is not only the leaves of this plant that has antioxidant potential, the fruit juice also possesses antioxidant properties that can be utilized.

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